LAL UPDATE®

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Dear LAL User,

This has been a year of great significance for Associates of Cape Cod, Inc. Dr. Watson's passing was a time of sadness and introspection for us all. An important part of this year's work has been adjusting to the loss of our founder. Fortunately, part of Dr. Watson's impressive legacy was a sound company based on quality products and staffed by dedicated people. It is now our task to build on this solid foundation. An important part of our future lies in our export sales. The reorganization of our distribution system in Europe this year has been a critical step. Our new subsidiary companies, ACCI Europe and ACCI UK have done outstanding work. Here in the US, we added to our technical staff and ran more training courses in 1995 than ever before. Because of the demand for training courses, we are adding a new training center onto our building. Another major facility improvement is a new bottling suite for multi-test vials which will be complete by mid-1996. Pyros™ for Windows™ software for the LAL-5000 has been very well received since its release earlier this year. We have a number of research and development irons in the fire and will keep you informed when new products are ready for market. We are looking forward to the challenges of 1996 and to an exciting future.

All of us at Associates of Cape Cod wish you and your families Happy Holidays and the very best for 1996.

Sincerely,

Thomas J. Novitsky, Ph.D.
Editor

Maximum Valid Dilution and Minimum Valid Concentration

by Michael E. Dawson, Ph.D.

The LAL test, regardless of method, is usually more sensitive than is necessary to detect the endotoxin limit of a product. Products can therefore be diluted to overcome interference while still allowing detection of the endotoxin limit. Dilution is by far the most important strategy for dealing with interference. However, a product may not be diluted beyond the point of being able to detect the endotoxin limit. This dilution is called the maximum valid dilution or MVD. The MVD is a dilution factor. An MVD of 20 represents a 1 part in 20 parts total dilution. The minimum valid concentration (MVC) is the product concentration at the MVD. It is important to know the MVD and/or MVC when confronted with a new product to test or validate.

The significance of the MVD is best illustrated by an example. If the endotoxin limit of product A is 5 EU/ml and the test sensitivity is 0.25 EU/ml, the method is clearly much more sensitive than is necessary to detect the limit. It is twenty times more sensitive than necessary. This factor of twenty is the MVD. If the product is contaminated with an endotoxin concentration of 5 EU/ml, it should fail the test. When this product is diluted by a factor of twenty, the endotoxin concentration in the dilution is 0.25 EU/ml, which is detectable and the product will fail the test. At greater dilutions, the endotoxin will not be detectable. A negative test at a dilution greater than the MVD gives no assurance that the product contains less than the endotoxin limit.

Conversely, a positive gel-clot test of undiluted product A using a reagent sensitivity of 0.25 EU/ml does not represent a failure. Such a test result only means that the product contains more than 0.25 EU/ml. It may still contain less than 5 EU/ml. In order to determine whether the product contains equal to or more than the limit concentration, it must be diluted to the MVD. It is for this reason that the test for Bacterial Endotoxins in the European Pharmacopeia (EP) states that samples should be tested at the MVD. Only when testing at the MVD are you performing a pass/fail test by the gel-clot method. (The EP and other pharmacopeia do not detail procedures for the turbidimetric or chromogenic methods).

Formulae for Calculation of MVD

The simplest formula for MVD applies if the endotoxin limit is expressed in EU/ml. This is the formula given in the EP, which refers to the endotoxin limit concentration (ELC) expressed in EU/ml. (See LAL UPDATE, June 1995 for discussion of endotoxin limits).

$$\mathbf{MVD} = \frac{\text{Endotoxin limit in EU/ml}}{\lambda}$$

where λ (lambda) is the sensitivity of the test (see box for details).

If the endotoxin limit is expressed in units other than EU/ml (e.g. EU/mg or EU/IU), the product concentration (or potency) must be included in the equation. This is the equation given in the FDA Guideline as Method I for calculating the MVD.

$$\mathbf{MVD} = \frac{\text{Endotoxin limit in EU/ml x product concentration}}{\lambda}$$

λ in Chromogenic and Turbidimetric Methods: A Moveable Feast

In the equations for MVD and MVC the term λ (lambda) is used to denote the sensitivity of the LAL test. In the case of the gel-clot test this is very simple; λ is the lysate sensitivity and is specific to the lot of LAL reagent. In the other methods, λ is not fixed. If a sample is found to contain no detectable endotoxin in an assay, the result is reported as less than (<) the concentration of the lowest standard. Thus, the sensitivity of any given test, i.e. λ, is the lowest concentration used to construct the standard curve for that assay. Because the range of the standard curve can be changed, so can λ.

When calculating MVD and MVC values for methods other than gel-clot, use for the value of λ the lowest concentration that you could employ in a standard curve. This will give the maximum possible value for the MVD (and the lowest possible MVC) for the method. Whether these extremes of MVD or MVC, or indeed of λ, are actually used is likely to depend on the degree to which the product interferes with the test. The degree of interference is determined in preliminary tests.

Example of MVD Calculation

To continue with the example given above in which product A has a limit of 5 EU/ml and the LAL has a sensitivity (λ) of 0.25 EU/ml:

$$\mathbf{MVD} = \frac{5 \text{ EU/ml}}{0.25 \text{ EU/ml}} = 20$$

Assume limit for product A is 0.05 EU/mg and the concentration is 100 mg/ml, then

$$\mathbf{MVD} = \frac{0.05 \text{ EU/mg x } 100 \text{ mg/ml}}{0.25 \text{ EU/ml}} = 20$$

It is important to note that MVD increases as the sensitivity of the test reagent increases. If the sensitivity of the LAL reagent used is 0.06 EU/ml for the above product (a reagent that is four times more sensitive) the MVD increases by a factor of four.

$$\mathbf{MVD} = \frac{5 \text{ EU/ml}}{0.0625 \text{ EU/ml}} = 80$$

Note the use of 0.0625 EU/ml in the calculation, even though the sensitivity of the LAL on the vial label is given as 0.06 EU/ml. The additional decimal places are meaningless in terms of LAL sensitivity because there is an allowance for a twofold error in the method. However, they do tend to yield round numbers. Indeed, if 0.06 is used for the divisor in the above calculations, we get 83.333 instead of a whole number. It is also a little more conservative to use the additional decimal places. This matter was discussed in the LAL UPDATE, December 1994.

While the MVD is the greatest dilution of product at which the endotoxin limit can be detected, the MVC is the lowest concentration of product at which the endotoxin limit is detectable in a given test method. Because it is a concentration, the MVC is directly applicable to products with endotoxin limits expressed in units such as EU/mg, EU/IU, EU/mEq, etc. It is of less value for products with endotoxin limits expressed in EU/ml, in which case the MVC is expressed in ml/ml.

While the MVD is a unitless dilution factor, the MVC has units of concentration. The most important distinction between the two is that, for a given test sensitivity, the MVC is absolute whereas the MVD is influenced by product concentration. Thus, two different potencies of a product will have different MVDs, but the MVC is constant. It is important to bear this in mind when making up test solutions from bulk powder; the MVD will depend upon the initial

concentration prepared. The greatest advantage of the concept of MVC is this independence from the initial product concentration.

A number of the pharmacopeia chapters on endotoxin tests refer to the MVD but none mention the MVC. Only the FDA Guideline refers to the MVC. Calculation of the MVC is the first step given to determine the MVD by method II. The following equation assumes that the endotoxin limit of the product has been determined, whereas the equation in the Guideline does not.

$$\text{MVC} = \frac{\lambda}{\text{endotoxin limit}}$$

Example of MVC Calculation

Again, we will use our hypothetical product A with an endotoxin limit of 0.05 EU/mg and an LAL sensitivity of 0.25 EU/ml:

$$\text{MVC} = \frac{0.25 \text{ EU/ml}}{0.05 \text{ EU/mg}} = 5 \text{ mg/ml}$$

The MVC is inversely related to the MVD. The greater the dilution of the product, the lower the concentration. The formula for converting an MVC to the MVD is:

$$\text{MVD} = \frac{\text{product concentration}}{\text{MVC}}$$

Example: Using the figures from our example:

$$\text{MVD} = \frac{100 \text{ mg/ml}}{5 \text{ mg/ml}} = 20$$

Because the MVC is inversely related to the MVD, an increased sensitivity of the test method reduces the MVC and increases the MVD. That is, the more sensitive test allows the endotoxin limit to be detected at a lower concentration/ greater dilution of the product.

In conclusion, while the MVC is more rugged than the MVD because it is independent of the initial product concentration, the concept of MVD is more universally applicable. An MVD can be calculated for any sample that has an endotoxin limit assigned to it. For calculation of MVDs, it is recommended that endotoxin limits be expressed in or converted to EU/ml (or IU/ml). (See the article on endotoxin limits in the LAL UPDATE, June 1995). This is the EP approach and it is a reasonable one because these are the units of test sensitivity and standards. Once the limit is in EU/ml, the concept of MVD becomes obvious. Use of the MVD requires an understanding of the concept, because an MVD is specific to a particular product concentration. It is important to appreciate this, otherwise invalid test results are possible.

We now have a web site where you can browse through our product catalog, the latest LAL UPDATE and listings of back issuses. Send us e-mail at: accinc@acciusa.com

Announcement

Change in Labeling of Pyrotell® LAL Single Test Vials

Starting in 1996, we will no longer be putting Mylar™ labels on **Pyrotell®** LAL single test vials (STVs). The vial will have the lot number printed directly on the glass by an ink jet dot printer of the type used to print expiration dates on many food containers. The package will still be labelled with the lot specific information: lot number, sensitivity, and expiration date. This change will increase our efficiency and give us better control of the packaging process and costs.